

REPLACEMENT SHEET

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RATIONAL DIRECTED PROTEIN EVOLUTION USING TWO-DIMENSIONAL RATIONAL MUTAGENESIS SCANNING



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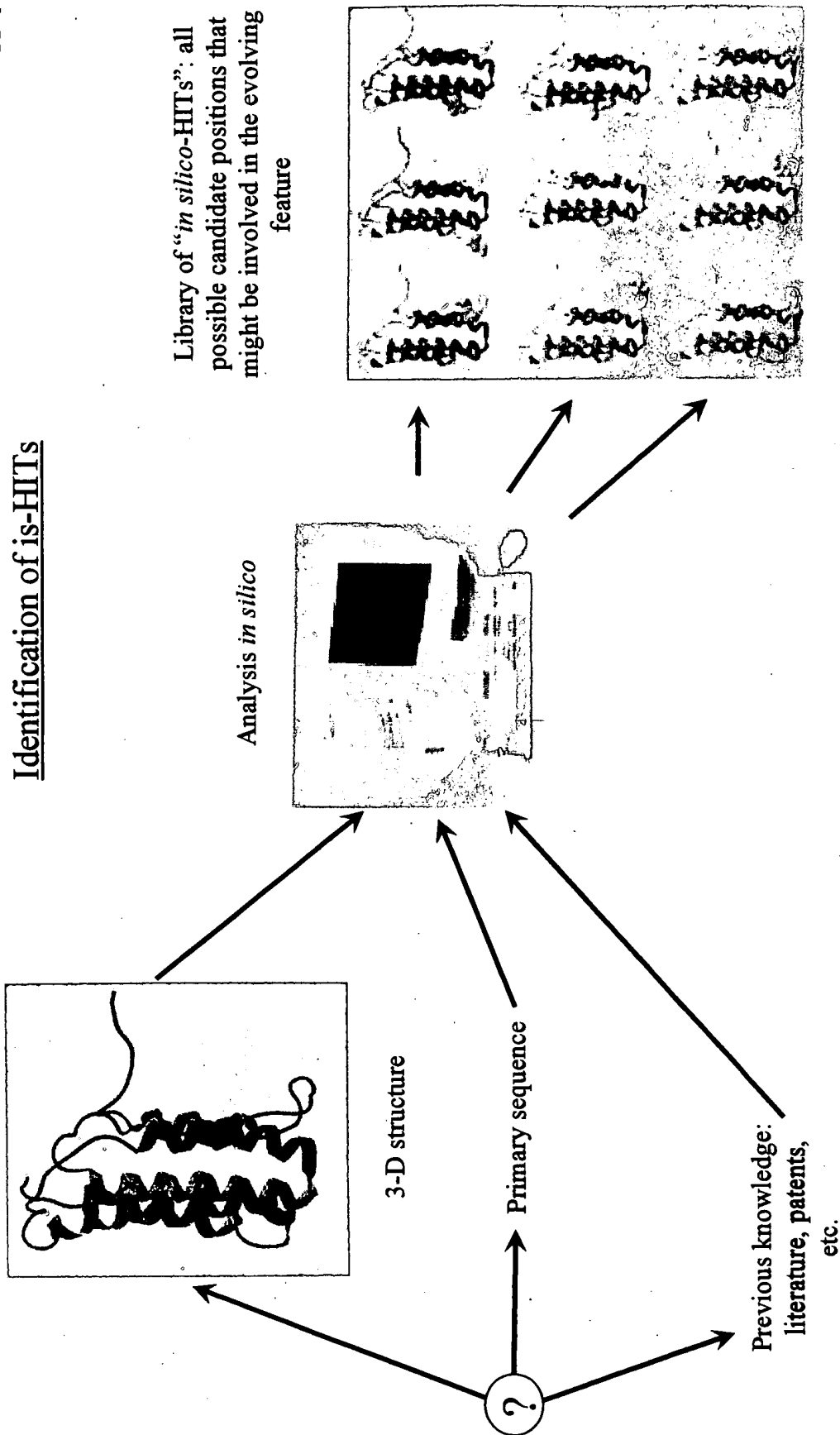


FIG.1A

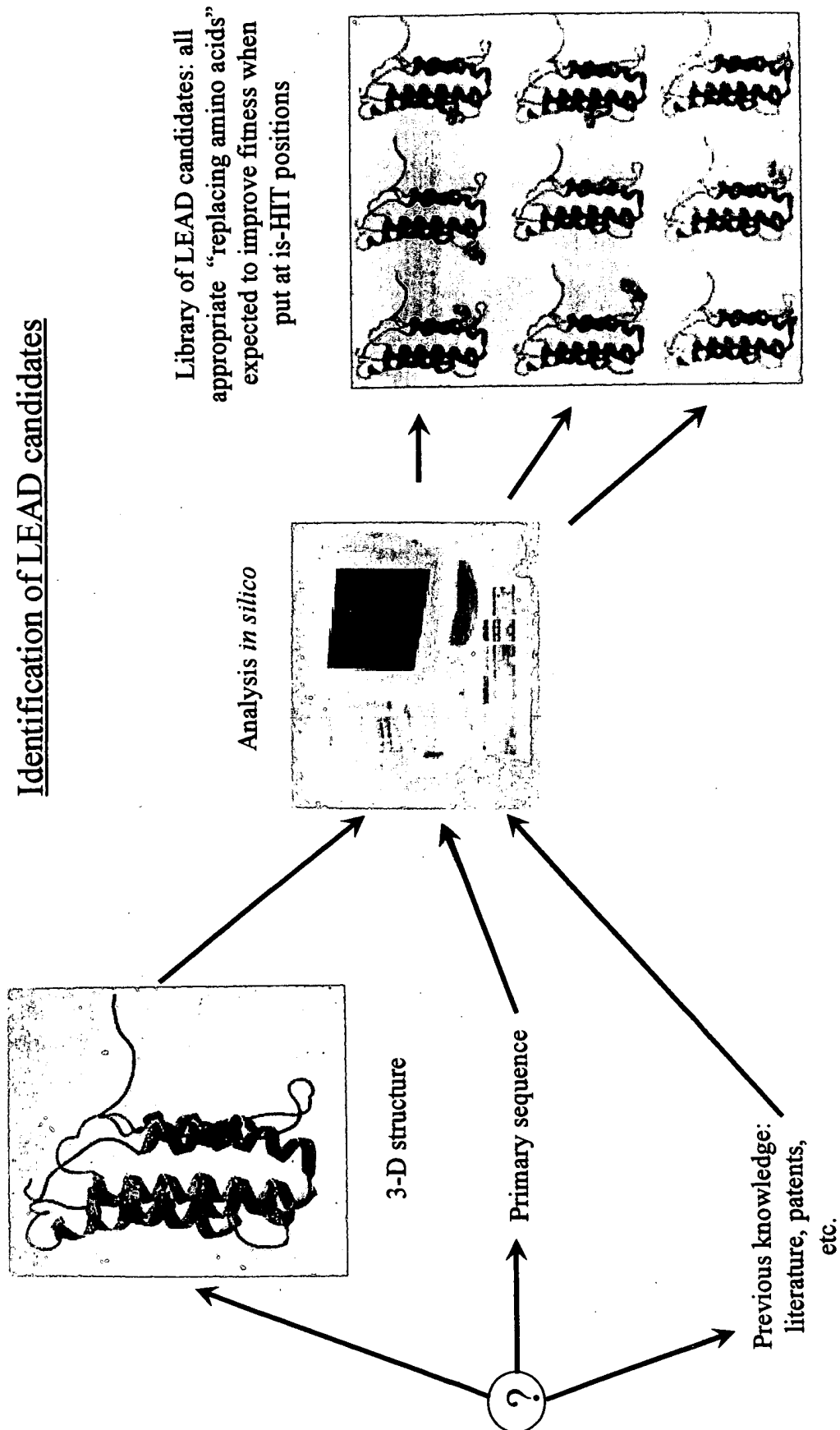


FIG.1B

Identification of LEADs : the optimized sequences at the is-HIT positions

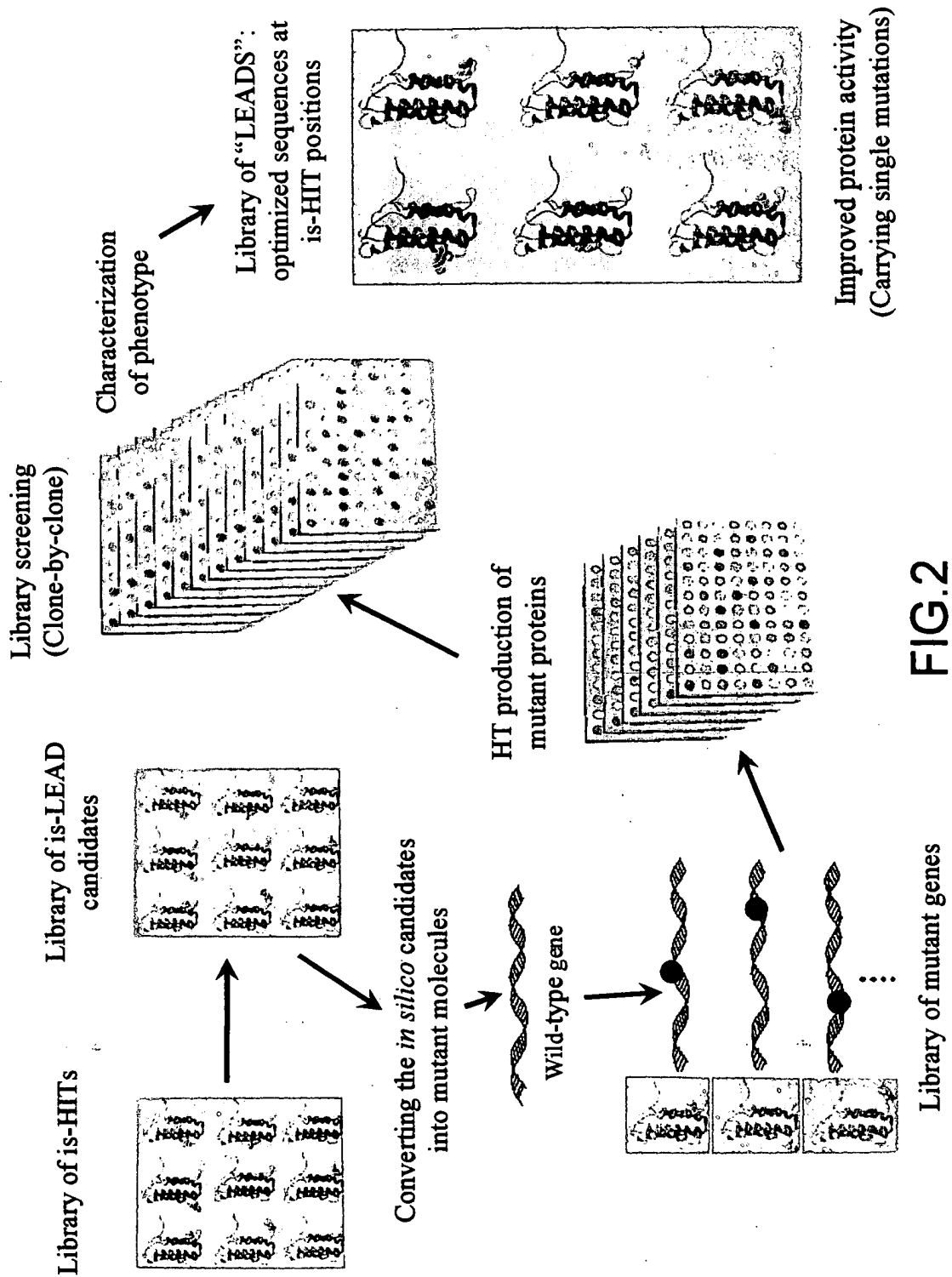
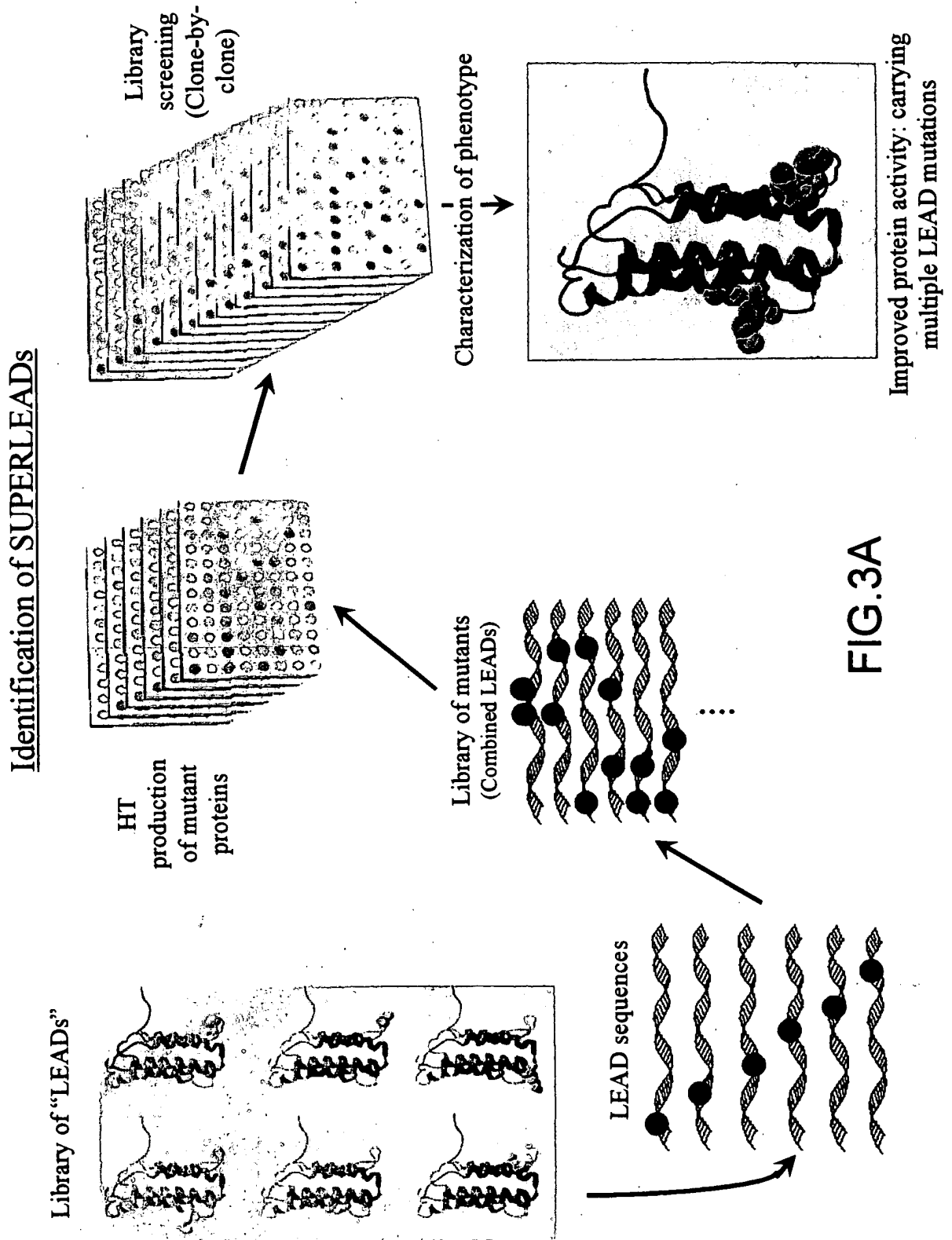
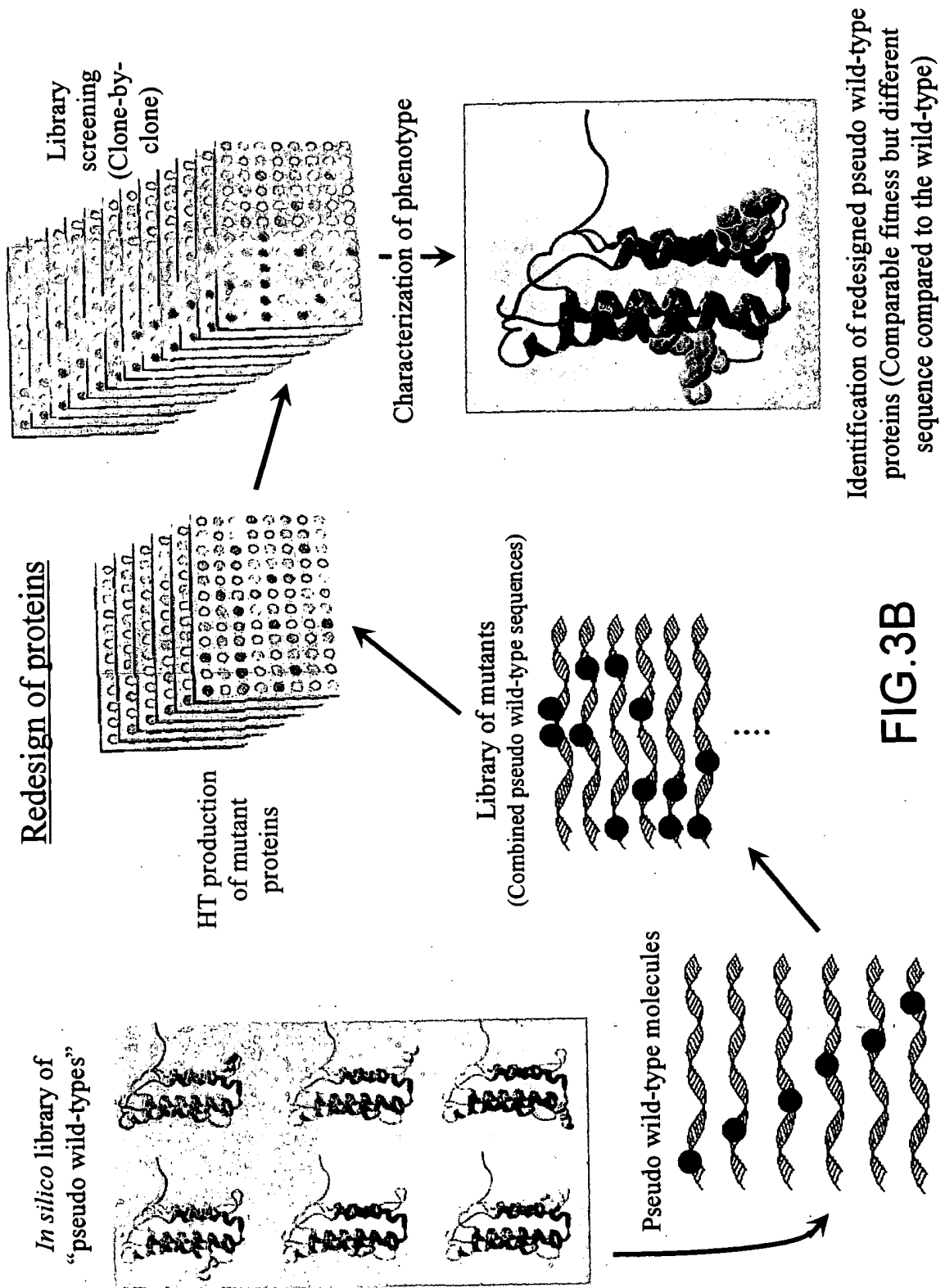


FIG.2



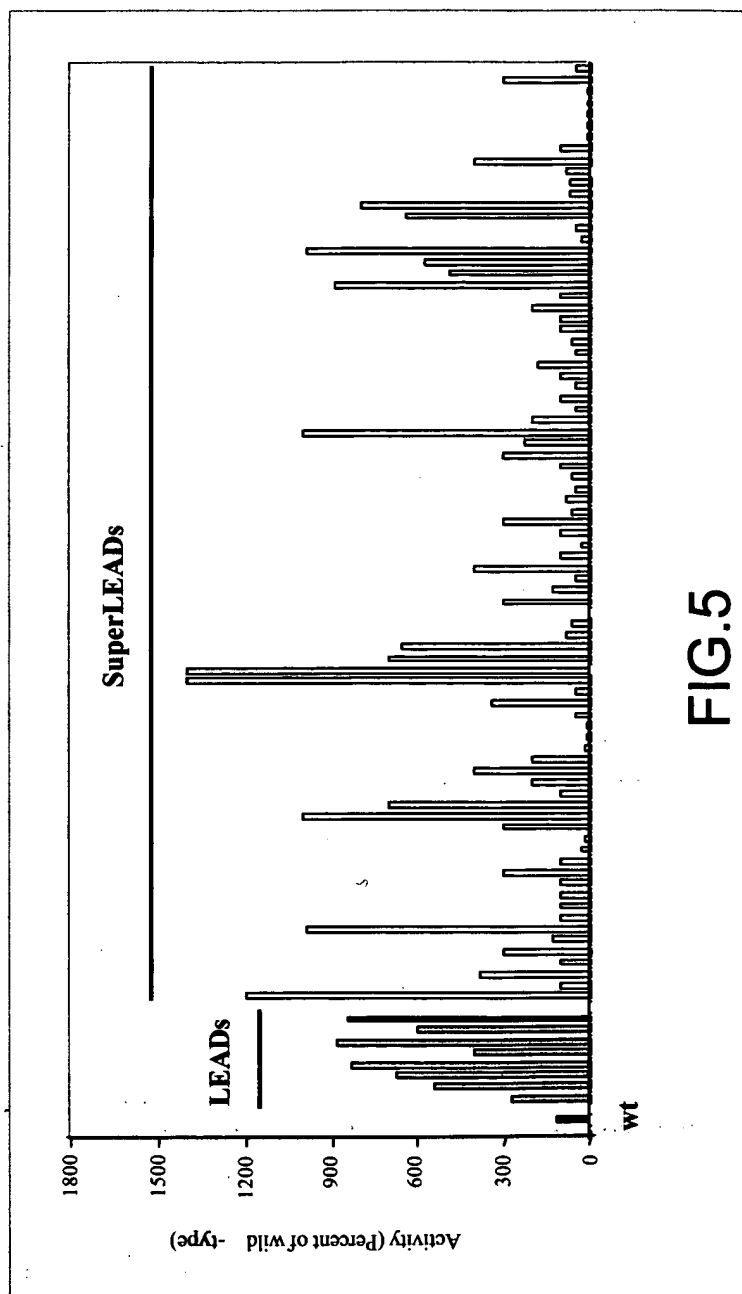


“Additive Directional Mutagenesis” (ADM)

CONSTRUCT	Mutation 1	Mutation 2	Mutation 3	Mutation 4	Mutation 5	Mutation 6	Mutation 7	Mutation 8	Mutation 9
Mutation 1	Mutation 2								
	Mutation 2	Mutation 3							
	Mutation 2	Mutation 3	Mutation 4						
	Mutation 2	Mutation 3	Mutation 4	Mutation 5					
	Mutation 2	Mutation 3	Mutation 4	Mutation 5	Mutation 6				
	Mutation 2	Mutation 3	Mutation 4	Mutation 5	Mutation 6	Mutation 7			
	Mutation 2	Mutation 3	Mutation 4	Mutation 5	Mutation 6	Mutation 7	Mutation 8		
	Mutation 2	Mutation 3	Mutation 4	Mutation 5	Mutation 6	Mutation 7	Mutation 8	Mutation 9	
	Mutation 2	Mutation 3	Mutation 4	Mutation 5	Mutation 6	Mutation 7	Mutation 8	Mutation 9	Mutation 10
Mutation 2		Mutation 3							
		Mutation 3	Mutation 4						
		Mutation 3	Mutation 4	Mutation 5					
		Mutation 3	Mutation 4	Mutation 5	Mutation 6				
		Mutation 3	Mutation 4	Mutation 5	Mutation 6	Mutation 7			
		Mutation 3	Mutation 4	Mutation 5	Mutation 6	Mutation 7	Mutation 8		
		Mutation 3	Mutation 4	Mutation 5	Mutation 6	Mutation 7	Mutation 8	Mutation 9	
		Mutation 3	Mutation 4	Mutation 5	Mutation 6	Mutation 7	Mutation 8	Mutation 9	Mutation 10
Mutation 3			Mutation 4						
			Mutation 4	Mutation 5					
			Mutation 4	Mutation 5	Mutation 6				
			Mutation 4	Mutation 5	Mutation 6	Mutation 7			
			Mutation 4	Mutation 5	Mutation 6	Mutation 7	Mutation 8		
			Mutation 4	Mutation 5	Mutation 6	Mutation 7	Mutation 8	Mutation 9	
			Mutation 4	Mutation 5	Mutation 6	Mutation 7	Mutation 8	Mutation 9	Mutation 10
Mutation 4				Mutation 5					
				Mutation 5	Mutation 6				
				Mutation 5	Mutation 6	Mutation 7			
				Mutation 5	Mutation 6	Mutation 7	Mutation 8		
				Mutation 5	Mutation 6	Mutation 7	Mutation 8	Mutation 9	
				Mutation 5	Mutation 6	Mutation 7	Mutation 8	Mutation 9	Mutation 10
Mutation 5					Mutation 6				
					Mutation 6	Mutation 7			
					Mutation 6	Mutation 7	Mutation 8		
					Mutation 6	Mutation 7	Mutation 8	Mutation 9	
					Mutation 6	Mutation 7	Mutation 8	Mutation 9	Mutation 10
Mutation 6						Mutation 7			
						Mutation 7	Mutation 8		
						Mutation 7	Mutation 8	Mutation 9	
						Mutation 7	Mutation 8	Mutation 9	Mutation 10
Mutation 7							Mutation 8		
							Mutation 8	Mutation 9	
							Mutation 8	Mutation 9	Mutation 10
Mutation 8								Mutation 9	
								Mutation 9	Mutation 10
Mutation 9									Mutation 10

FIG.4

LEADs and SuperLEADs obtained for the Rep protein



Amino acid sequence of human mature IFN α -2b

IFN α -2b 1 10 20 30 40 50

 CDLPQTHSLGSRRTIMLLAQMRRISLFSCLKDRHDFGFPQEEFGNQFQKA

IFN α -2b 51 60 70 80 90 100

 ETIPVLHEMIQQIENLFSTKDSSAAWDETLLDKFYTELYQQQLNDLEACVI

IFN α -2b 101 110 120 130 140 150

 QGVGVTEPTLMKEDSILAVRKYFORITLYLKEKKYSPCAWEVVRAEIMRS

IFN α -2b 151 160
 . .
 FSLSTNLQESLRSKE

FIG.6A

Three dimensional structure of INF α -2b

showing candidate LEADs



FIG.6B

The "Percent Accepted Mutation" (PAM250) matrix

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	2	-2	0	0	-2	0	0	1	-1	-1	-2	-1	-1	-3	1	1	1	-6	-3	0
R	-2	6	0	-1	-4	1	-1	-3	2	-2	-3	3	0	-4	0	0	-1	2	-4	-2
N	0	0	2	2	-4	1	1	0	2	-2	-3	1	-2	-3	0	1	0	-4	-2	-2
D	0	-1	2	4	-5	2	3	1	1	-2	-4	0	-3	-6	-1	0	0	-7	-4	-2
C	-2	-4	-4	-5	12	-5	-5	-3	-3	-2	-6	-5	-5	-4	-3	0	-2	-8	0	-2
Q	0	1	1	2	-5	4	2	-1	3	-2	-2	1	-1	-5	0	-1	-1	-5	-4	-2
E	0	-1	1	3	-5	2	4	0	1	-2	-3	0	-2	-5	-1	0	0	-7	-4	-2
G	1	-3	0	1	-3	-1	0	5	-2	-3	-4	-2	-3	-5	0	1	0	-7	-5	-1
H	-1	2	2	1	-3	3	1	-2	6	-2	-2	0	-2	-2	0	-1	-1	-3	0	-2
I	-1	-2	-2	-2	-2	-2	-2	-3	-2	5	2	-2	2	1	-2	-1	0	-5	-1	4
L	-2	-3	-3	-4	-6	-2	-3	-4	-2	2	6	-3	4	2	-3	-3	-2	-2	-1	2
K	-1	3	1	0	-5	1	0	-2	0	-2	-3	5	0	-5	-1	0	0	-3	-4	-2
M	-1	0	-2	-3	-5	-1	-2	-3	-2	2	4	0	6	0	-2	-2	-1	-4	-2	2
F	-3	-4	-3	-6	-4	-5	-5	-5	-2	1	2	-5	0	9	-5	-3	-3	0	7	-1
P	1	0	0	-1	-3	0	-1	0	0	-2	-3	-1	-2	-5	6	1	0	-6	-5	-1
S	1	0	1	0	0	-1	0	1	-1	-1	-3	0	-2	-3	1	2	1	-2	-3	-1
T	1	-1	0	0	-2	-1	0	0	-1	0	-2	0	-1	-3	0	1	3	-5	-3	0
W	-6	2	-4	-7	-8	-5	-7	-7	-3	-5	-2	-3	-4	0	-6	-2	-5	17	0	-6
Y	-3	-4	-2	-4	0	-4	-4	-5	0	-1	-1	-4	-2	7	-5	-3	-3	0	10	-2
V	0	-2	-2	-2	-2	-2	-2	-1	-2	-4	2	-2	2	-1	-1	-1	0	-6	-2	4

FIG.7

Scores from PAM250, given to residue substitutions to protect human INF α -2b against proteolysis

	R	D	E	L	K	M	F	P	W	Y
A	-2	0	0	-2	-1	-1	-3	1	-6	-3
N	0	2	1	-3	1	-2	-3	0	-4	-2
C	-4	-5	-5	-6	-5	-5	-4	-3	-8	0
Q	1	2	2	-2	1	-1	-5	0	-5	-4
G	-3	1	0	-4	-2	-3	-5	0	-7	-5
H	2	1	1	-2	0	-2	-2	0	-3	0
I	-2	-2	-2	2	-2	2	1	-2	-5	-1
S	0	0	0	-3	0	-2	-3	1	-2	-3
T	-1	0	0	-2	0	-1	-3	0	-5	-3
V	-2	-2	-2	2	-2	2	-1	-1	-6	-2

FIG.8

Residue substitutions expected to allow the
creation of a disulfide bond



FIG.9A



FIG.9B

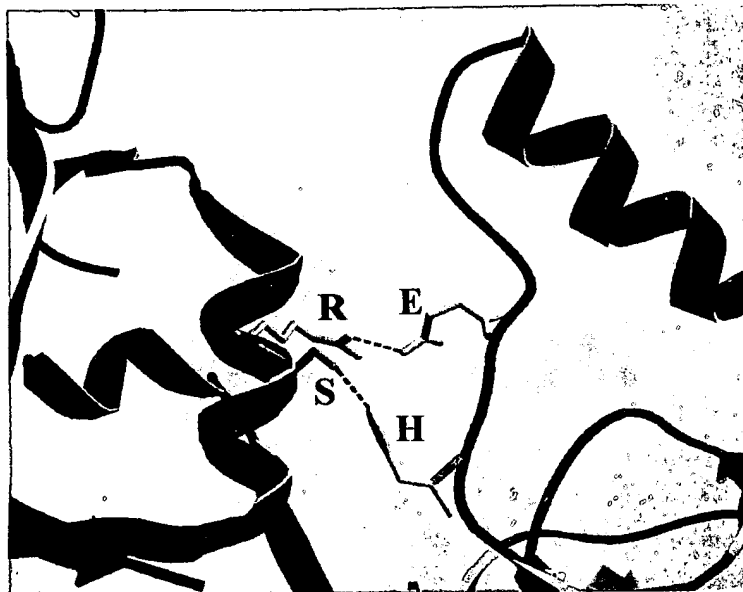
Residue substitutions expected to destroy linking interactions

FIG.10A



FIG.10B

Tri-dimensional model of an amphipathic polypeptide

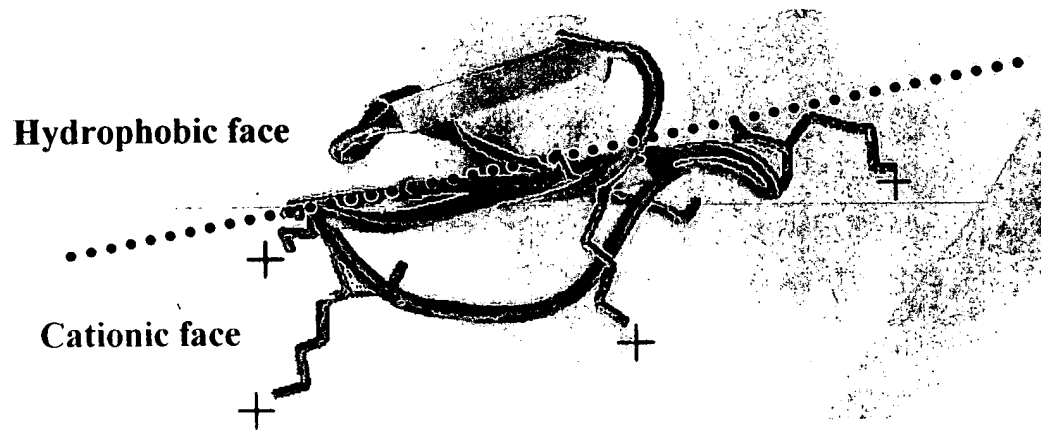


FIG.11

2-D matrix representation of a protein sequence

MVGESVPAVDQVLGTSKIYNP VNAIWDTIRN WRAHV NPF

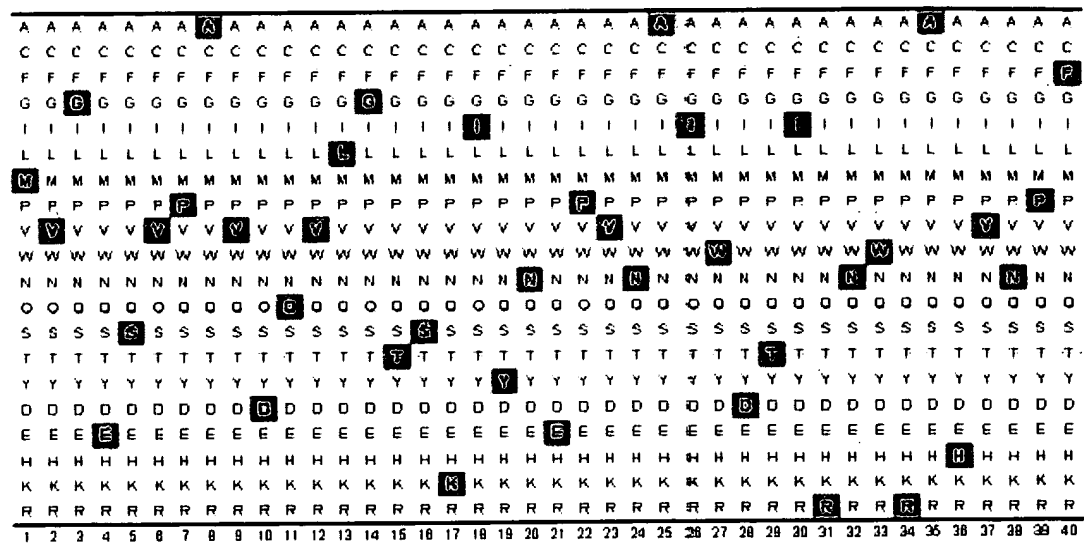


FIG.12

2-D matrix for amphipathic peptide showing K/R scanning; sequential replacement of each residue by either K or R

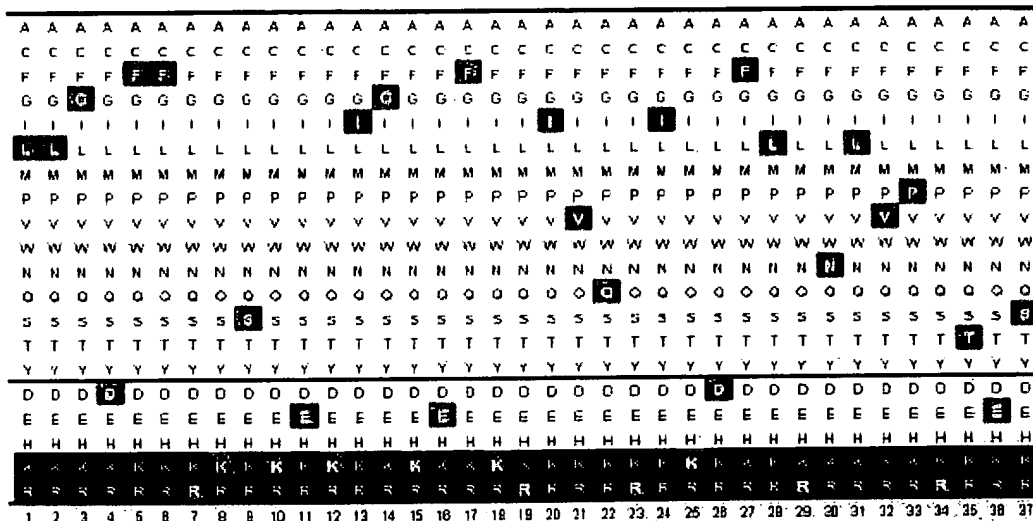


FIG.13A

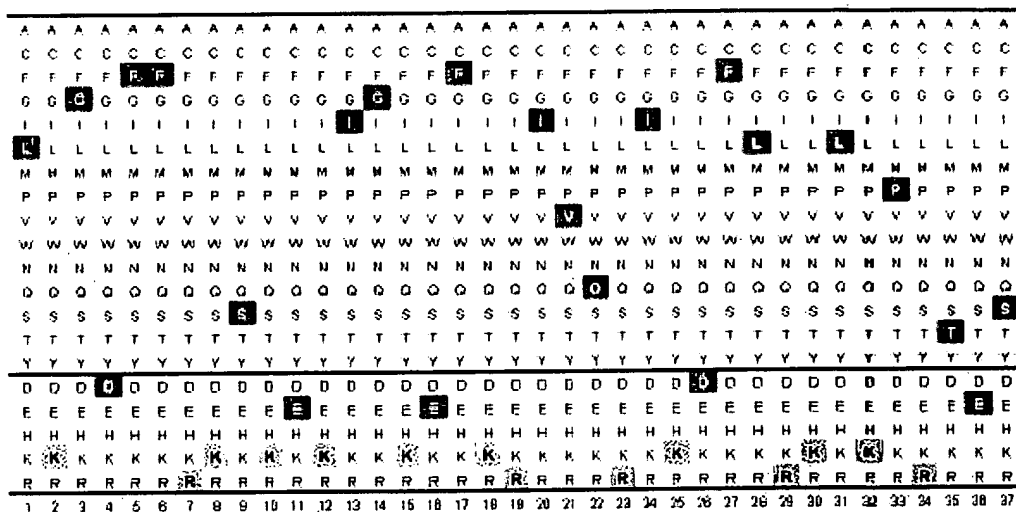


FIG. 13B

2-D matrix for LEAD candidates on amphipathic polypeptide

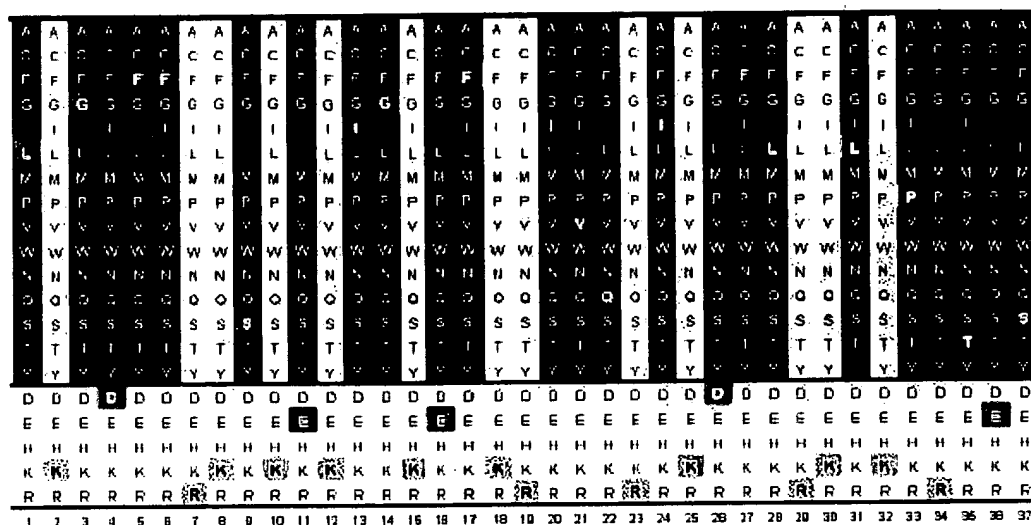


FIG. 13C

2-D matrix for optimized amphipathic polypeptide, following both: *i*) K/R scanning (FIG. 13B) and *ii*) mutagenesis (FIG. 13C)

[illegible]

FIG. 13D

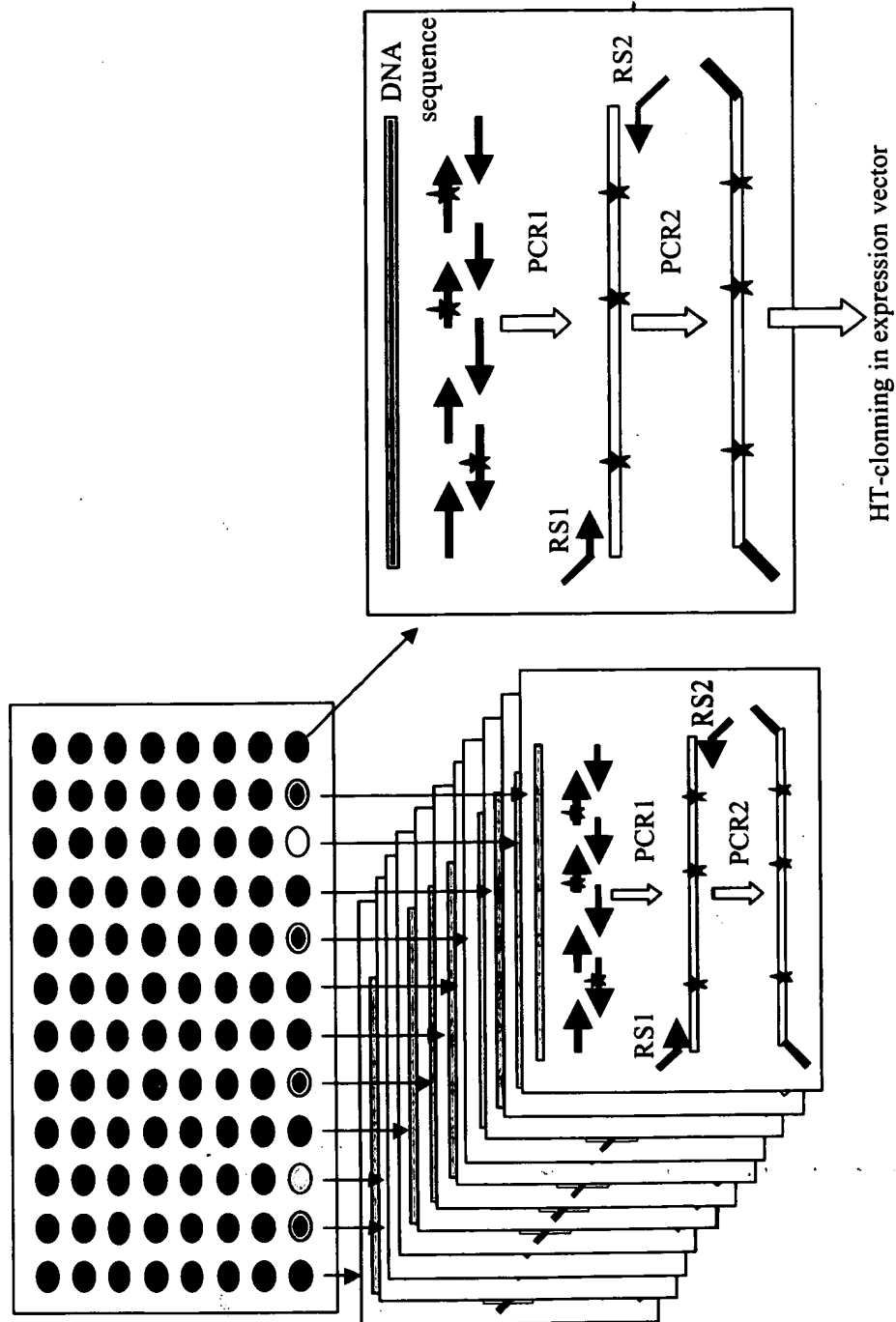
Multi overlapped primer extensions for rational recombination of LEADs

FIG.14